What Kind of Fish is That?

Have you sometimes wondered if that "wild caught" salmon actually came from an aqua farm? Or if the "U.S. catfish" in the display case might have been born and raised in Vietnam?

Is that "red snapper" actually red snapper and worth the premium price?

Scientists at the Food and Drug Administration (FDA) are able to answer those questions through a project that creates DNA barcodes to identify individual fish species. The massive project is part of an effort aimed at solving the problem of species substitution.

Species substitution can result in cheap fish being labeled as pricy ones, but mislabeling can also threaten public health. For example, in 2007, a prohibited and highly toxic variety of puffer fish, also known as fugu or blowfish, was smuggled into the U.S. in boxes labeled as "headless monkfish." This deception resulted in illnesses in multiple states.

A series of cutting-edge tests must be conducted to create the barcodes, which look much like the lines of different thicknesses on Universal Product Code (UPC) labels used to identify and scan manufactured products. However, unlike the barcodes you see on packages in the supermarket, the barcodes that identify different fish species will not be attached to the fish.

Instead, once a fish species is identified through DNA testing and other high-tech techniques in FDA labs, the newly created barcode unique to that species is entered into a database, which could be thought of as a library or catalogue of commercial fish species.

When encountering a fish or fish product (fillets, fish sticks, sushi, etc.) whose species is unknown, inspectors with the equipment and know-how can create a barcode for that fish and compare it against FDA's database to seek a known match.

The agency has trained more than 20 FDA analysts around the country to use that procedure in many of its regional field laboratories and are now performing the analysis on a regular basis.

FDA research biologist Jonathan Deeds, Ph.D., is working to assure that U.S. seafood is both safe and accurately labeled. DNA from fish such as these are being used as part of a new testing program to enforce the accurate labeling of seafood sold in the U.S. For high resolution copyright-free photos of this project available for redistribution, go to: *www.flickr.com/photos/fdaphotos/sets/72157638253069273/.*

Collecting Samples

The first step in FDA's species identification project involves collecting fresh fish to be tested.

Jonathan Deeds, Ph.D., an FDA research biologist, has been showing up at fishing tournaments and seafood conventions in the U.S., asking for donations of fish he can bring back to suburban Maryland for testing.

Meanwhile, Jeffrey Williams,

Ph.D., has led three expeditions to markets in the Philippines, collecting nearly a thousand fish to be used by the FDA for species testing. FDA contracted with the Smithsonian Institution's Division of Fisheries, and Laboratories of Analytical Biology for their expertise in taxonomy—the naming of species, and for their expertise in storing species long term.

The Smithsonian already has the world's largest fish collection, started in the 1800s. However, modern genetic tests cannot be performed on fish stored using standard museum practices of preserving with formaldehyde. By collecting new species, Smithsonian scientists like Williams are doing work critical to FDA's species substitution project, but are also serving their mission of building the national fish collections.

When a fresh fish arrives at FDA labs, Deeds removes a small piece. He turns this over to FDA molecular biologist Sara Handy, Ph.D., to test its DNA—the nucleic acid that carries genetic information. Deeds saves a larger chunk in case future DNA sampling is needed. The piece is stored for long-term use in freezers at ultra-low temperatures of -80 degrees Celsius.

The remainder of the whole fish is sent to Williams and his team at the Smithsonian for authentication and indefinite safekeeping.

As a final step, FDA information technology specialist Frederick Fry, Jr., Ph.D., has created a public database of barcodes for commercial seafood, available through the FDA website, that is used by regulators both inside and outside the FDA, by private laboratory scientists on behalf of seafood suppliers, and by academic researchers around the world.

The Need Is Critical

While the technology is still evolving, the ability to prevent mislabeling is increasingly critical. Worldwide, about 30,000 species of fish are thought to exist, about 1,500 of which currently are sold commercially in the U.S. As we run out of the most popular fish types, the number of species harvested is expected to increase. That in turn will likely lead to more confusion about what's being bought and sold.

Although new species are still being added to the database, DNA evidence has already been used by FDA in support of enforcement actions against fish wholesalers found to be substituting one fish for another. In 2013, for example, the Department of Justice charged the owner of an Illinois seafood distributor with mislabeling fish. The owner subsequently pleaded guilty and received a maximum $100,000 fine and was sentenced to five years' probation.

It also has been used to test and reject imported fish that are misbranded, and has helped investigators trace the source of illness outbreaks. DNA testing can pinpoint with much greater accuracy what kind of fish was involved in an outbreak. For example, scientists may suspect that the source of an illness was a "snapper" or a "grouper." But there are more than 100 species that can be marketed within those two categories alone. Being able to determine the exact species involved in an illness provides critical clues to finding and eliminating the source of the problem.

Scientists at the Canadian Centre for DNA Barcoding have proposed creating a portable barcoding device so that this process can move beyond the laboratory.

Previously, to identify a species the FDA relied on a combination of physical characteristics and a protein analysis that wasn't as exact and that didn't work well on fish that had been cooked. Moreover, protein in the fish broke down over time, so there was a constant need to refurbish fresh fish samples. Moreover, making comparisons between the fish in question and reference samples was difficult and time consuming.

The Science of Identifying Species

The fish barcoding project demonstrates the advanced science practiced in FDA labs. Procedures include some similar to those used to create the human genome, a genetic map of the body.

The DNA of all living things is made up of just four chemical bases—adenine (A), guanine (G), cystosine (C), and thymine (T). Millions of those four bases are present in an organism as complex as a fish. The order, or "sequence," of those bases is unique to every species.

Sequencing all the DNA in a fish sample would provide too much information—specifically, the order of all those millions of bases that make up the DNA of a fish. Instead, scientists can now identify one specific piece of DNA that has just 650 bases. That fragment typically provides enough genetic information to identify a fish species.

Requiring only a pinhead-sized piece of fish tissue, that specific piece of DNA is isolated and replicated, in other words copying it and separating it from all of the other DNA, using a technique called Polymerase Chain Reaction or PCR. The PCR technique, performed on an instrument called a thermocycler, can isolate this particular fragment of DNA from nearly all fish species. Several other steps, including analyzing with a DNA sequencer, will eventually lead to a unique bar code for each species.

The technique can be used to identify anything from skinned fish fillets to tiny bits of fish in a cooked soup.

"We know that our team's DNA project has immediate practical applications to prevent seafood fraud and increase the safety of seafood," says Deeds. "The additional benefits in the future to science and the public we can only imagine."

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